Design and Evaluation by Nitrogen Balance and Blood Aminograms of an Amino Acid Mixture for Total Parenteral Nutrition of Adults with Gastrointestinal Disease

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ABSTRACT This study was undertaken to assess the efficacy of an amino acid mixture formulated for intravenous use from estimates of requirements for essential amino acids of human adults, and from data previously derived from a study using casein hydrolysate as the amino acid source. This mixture contained 39.4% essential amino acids, with glycine, alanine, arginine, histidine, and proline selected to supply the nonessential nitrogen.

Nitrogen balance and blood aminograms were measured in six adult patients with gastrointestinal disease, fed intravenously for 1 wk at each of three levels of amino acid intake, while all other nutrients were constant and adequate in relation to body weight. Four of the patients were then fed 1.0 g/kg of egg or meat protein orally for 1 wk at the end of the 3 wk study, while all nutrients other than the amino acids were fed intravenously. Average nitrogen balances of − 0.8, 0.5, and 2.2 g/day were observed when the amounts of amino acids infused were 0.25, 0.5, and 1.0 g/kg, respectively. Fasting levels of threonine, glycine, cystine, methionine, isoleucine, tryptophan, and arginine, but not of the other amino acids, increased with the increment in input, although the levels were generally lower than normal. The increase in blood concentration observed during infusion was similar for each of the essential amino acids, indicating that the supply of each of the amino acids was much better balanced for utilization than casein hydrolysate. However the blood aminograms did suggest that some changes in composition of the mixture for the malnourished adult were desirable, such that the amino acid mixture resembled that needed by the normal growing child. Nitrogen balance in the four patients fed orally with high-quality protein averaged 2.0 g/day, showing that an amino acid mixture given intravenously, provided it is well-balanced, can be utilized as efficiently as protein given orally. It is concluded that the prediction of oral requirements for amino acids should be combined with observations on changes in blood aminograms during infusion over a range of amino acid input in the development of amino acid mixtures for intravenous feeding.

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INTRODUCTION

Previous studies in which casein hydrolysate was used as the nitrogen source in total parenteral nutrition of malnourished adults with gastrointestinal disease indicated that nitrogen equilibrium could be sustained with about 0.8 g of hydrolysate/kg of body weight (1). However, analyses of blood aminograms indicated that casein hydrolysate was imbalanced in its amino acid composition and contained a relative deficiency of methionine, cystine, phenylalanine, and tyrosine, but an excess of valine and lysine. Further evidence that casein hydrolysate given intravenously or orally was not an ideal nitrogen source was obtained from nitrogen balance data that showed that nitrogen retention was much greater when an equivalent amount of protein was given orally as cottage cheese. Therefore we postulated that an amino acid mixture formulated in accordance with the most recent estimates of the requirements of the human adults (2) would be a more reasonable nitrogen source for parenteral nutrition. The present study was undertaken primarily to assess the efficacy of such an amino acid mixture. A secondary objective was to assess the relationship of oral requirements to intravenous requirements for amino acids by studying blood aminograms by the methods previously described (1).

METHODS

Patients studied. Six adult patients with various gastrointestinal disorders were studied (Table I).

Experimental design. The patients were admitted to the Clinical Investigation Unit of the Toronto General Hospital, where they were fed intravenously through a subclavian catheter. All patients were given 55 kcal/kg body weight, excluding the amino acid calories, plus all nutrients except amino acids, in adequate and constant proportions to body weight. The total of amino acids infused was varied so that the solutions supplied either 0.25, 0.5, or 1.0 g of amino acids/kg body weight. Half the nonprotein calories were given as carbohydrate and half as lipid (Intralipid, Vitrum Laboratories, Stockholm, Sweden). Details of the solutions infused are given in Table II.

The solutions containing carbohydrate, amino acids, vitamins, and electrolytes were infused by gravity drip between 0900 and 2100 h, while between 2100 and 0900 h lipid was infused. Each concentration of the amino acids was given for 7 days, so that each patient was on this study for a total of 3 wk. Four of the patients (S., M., G., and W.) received the amino acids in increasing concentration while two (T., K.) received the amino acids in decreasing concentration. At the end of the 3-wk intravenous study, patients S., T., G., and W. were given 1 g/kg of protein orally in the form of eggs or meat for 7 days in six equal meals while the same nonprotein calories, but no amino acids, were fed intravenously. The first oral meal of protein was given at 0900 h and the last at 2000 h. After 2 days of equilibrium at each amino acid or protein intake, daily urines were collected for 5 days and 100-ml aliquots were frozen for nitrogen estimations. Aliquots of three- and two-day stool and fistula drainage collections were also taken for nitrogen estimation. On the 7th day of infusion at each amino acid level, blood samples were collected at 0900 and 2100 h in heparinized tubes for amino acid analysis. The first sample was taken at the end of lipid infusion, 12 h after amino acid infusion had ended. The other sample was taken just before the end of the amino acid infusion at 2100 h. When the patients were fed protein orally, the 0900 h sample was taken before the first protein meal and the 2100-h sample was taken 1 h after the last meal of protein.

Whole blood, rather than plasma, was analyzed in these and the previous studies (1) because the data of Elwyn (3) and Aoki, Brennan, Muller, Moore, and Cahill, (4) suggest that erythrocyte or whole blood concentrations of amino acids were more useful than plasma amino acids as indicators of amino acid uptake into tissues and protein. Also, it had been shown by Snyderman, Holt, Norton, and Phansalkar (5) that when the amount of dietary protein was altered, the amino acid levels changed proportionately in plasma and red blood cells.

Electrolytes and blood urea nitrogen were analyzed twice a week, and serum glutamic oxaloacetic transaminase, alkaline phosphatase, serum proteins, calcium, magnesium, and phosphorus analyses were performed once a week.

TABLE I

Patient Description

Patient	Sex	Age	Height	Weight	Diagnosis
		yr	*cm*	*kg*	
S.	M	66	168	42	Calcific pancreatitis.
M.	M	64	178	70	Emergency gastrectomy for bleeding ulcer with dehiscence of wound and fistula.
T.	F	59	150	35	Total gastrectomy for Zollinger-Ellison syndrome with fistula.
K.	F	27	168	46	Short gut syndrome.
G.	F	24	160	34	Crohn's disease with enterocutaneous fistula.
W.	F	60	155	30	Post-gastrectomy malnutrition.

TABLE II

Details of Nutrients Infused when the Total Amino Acid Infused was 0.25, 0.50, or 1.00 g/kg body wt

Solution*	50% Glucose‡,¶	10% Amino acids§,¶	Water	10% Intralipid‖	Total		
					Fluid	Amino acids	Nonprotein
	ml	*ml*	*ml*	*ml*	*ml*	*g*	*kcal*
A	15	2.50	32.50	27	77	0.25	55
B	15	5.00	30.00	27	77	0.5	55
C	15	10.00	25.00	27	77	1.0	55

* Solution A, B, or C was infused to provide the desired input of 0.25, 0.50, or 1.00 g/kg of amino acids.

‡ Glucose monohydrate (3.4 kcal/g) as 50% (wt/vol) solution.

§ 10% Travesol Amino Acid Injection (Travenol Laboratories, Artificial Organs Div., Morton Grove, Ill.).

‖ 10% Intralipid = 1100 kcal/1000 ml.

¶ This mixture of glucose and amino acid also contained electrolytes, vitamins, and trace metals; Electrolytes (meq/day): Na, 100–120; K, 80–90; Ca, 20–22; Mg, 19–22; PO_4, 40–48. Remaining anions as chloride and lactate in ratio of 7:1. Vitamins: 5 ml/day MVI 1,00 (Arlington Laboratories, Montreal, Canada) provided the following: ascorbic acid, 500 mg; vitamin A, 5,000 IU; vitamin D, 500 IU; thiamine, 25 mg; riboflavine, 5 mg; niacinamide, 50 mg; pyridoxine, 7.5 mg; *d*-panthenol, 12.5 mg; vitamin E, 5 IU. The following were added once weekly: folic acid, 15 mg; vitamin K (Synkayvite, Hoffman-La Roche Ltd., Montreal, Canada), 10 mg; vitamin B_{12} (Rubramin, E. R. Squibb & Sons, Montreal, Canada), 75 μg. Trace metals (daily): Zinc, 200 μg; cobalt, 8 μg; manganese, 20 μg; copper, 20 μg; iodine, 120 μg.

TABLE III
Composition of Amino Acid Mixture

Essential*		Nonessential	
	mg/g		
Threonine	42	Histidine	44
Valine	46	Glycine	208
Methionine	58	Alanine	208
Isoleucine	48	Arginine	104
Leucine	62	Proline	42
Phenylalanine	62	Tyrosine	4
Lysine	58		
Tryptophan	18		
Total	394		610

* 39.4% of total.

The basic amino acids were added in the chloride form. However, the whole mixture was buffered by the addition of acetate.

Since no change occurred in these measurements during the study, the data are not reported here.

Composition of the amino acid mixture. The composition of the amino acid mixture used in these studies is shown in Table III.

Determination of nitrogen and blood amino acid. The nitrogen content of duplicate aliquots of urine and stool was determined by a micro-Kjeldhal method (6). The blood samples were prepared for amino acid analyses by precipitation with an equal volume of cold 20% trichloroacetic acid (TCA),[1] followed by two extractions with 10% TCA. After the TCA was extracted with ether, the combined supernate was freeze-dried. The amino acids were taken up in an appropriate volume of pH 2.2 lithium citrate buffer before analysis by standard procedures (7). Tryptophan in the blood samples was estimated by the method of Hess and Udenfriend (8).

Calculation of change in blood amino acid levels during infusion. The results were analyzed in two ways: first by a modification of the method utilized by Young, Hussein, Murray, and Scrimshaw (9), and Young, Tontisirin, Ozalp, Lakshmanan, and Scrimshaw (10). These investigators showed a sudden rise in fasting plasma amino acid level when the input of the amino acid under study met the requirements of human volunteers fed adequate and constant amounts of all other amino acids. During these present studies with parenteral infusion, the time equivalent to fasting was before the start of the amino acid-carbohydrate mixture at 0900 h each day. Hence we measured changes in "fasting" blood amino acid concentration with increasing input of amino acids. This change was evaluated for statistical significance by subtracting the 0900-h blood amino acid levels observed during the infusion of 0.25 g/kg of amino acids from that observed during the infusion of 0.5 and 1.0 g/kg in individual patients. Similarly, the 0900-h blood amino acid level observed during the infusion of 0.5 g/kg of amino acids was subtracted from that observed during infusion of 1.0 g/kg in individual patients. These differences were tested with the Walsh test (11, 12) appropriate for small sample size. In this way each patient was compared to himself or herself and thus variation in blood amino acid levels between different patients did not mask changes in the individual with increasing input of amino acids.

The second method was designed to observe the increment of blood amino acid above the fasting level during dierent rates of infusion. It is clear that a significant and progressive increment occurring with an increase in input of amino acid would indicate an expansion of the free amino acid pool, which in turn would suggest that supply of that amino acid exceeded utilization. This calculation was made by subtracting the preinfusion 0900-*h* values, for 0.25 g/kg, from the 2100 h value obtained after the infusion for 12 h of 0.25 and 1.0 g/kg of amino acids. The difference between the rise in blood amino acids above the 0.25 *g* fasting level at the end of the 12 h infusion period with 0.25 and 1.0 g/kg of amino acids was calculated for the individual patients. Again the Walsh test was used to determine statistical significance. A significant elevation was considered to indicate that the input exceeded the utilization of the amino acid concerned and therefore had met the requirements for protein synthesis and other metabolic functions.

RESULTS

Nitrogen balance studies. The data are shown in Table IV. A mean positive nitrogen balance of 0.5 g/day was obtained with an infusion of 0.5 g/kg of amino acids. When 1 g/kg of amino acids was given, the average nitrogen retained was 2.2 g/day. This latter amount did not differ from the 2.0 g of nitrogen retained when 1 g/kg of protein was given orally to the same patients.

From Table V it is clear that urinary nitrogen excretion at each level of nitrogen intake fluctuated around the mean over the 5-day collection period, and there was no upward or downward trend suggesting that the patients were not in nitrogen equilibrium. Furthermore, the effect of levels of nitrogen input on urinary nitrogen excretion was similar in patients infused first with either

TABLE IV
Nitrogen Balance with Intravenous Amino Acid and Oral Protein

Patient	Total amino acids infused			Oral protein
	0.25 g/kg	0.5 g/kg	1.0 g/kg	1.0 g/kg
		g/day		*g/day*
S.	−1.1	−0.1	2.3	3.7
M.	0.1	2.0	2.2	—
T.	−0.4	0.3	1.3	1.7
K.	−0.7	0.8	3.4	—
G.	−0.9	0.0	1.8	1.9
W.	−1.7	−0.1	—	1.6
Mean ±SEM	−0.8±0.2	0.5±0.3	2.2±0.3	2.0±0.6

[1] *Abbreviation used in this paper:* TCA, trichloroacetic acid.

TABLE V

Urinary Nitrogen during Nitrogen Balance Study Period of Patients Fed 0.25, 0.5, or 1.0 g/kg N Intravenously or 1.0 g/kg Orally

Day	Patient T.	G.	W.	S.	K.	M.
			g/24 h			
0.25 g/kg i.v.						
3	1.8	1.7	3.3	2.6	1.8	3.0
4	1.5	2.0	3.1	1.9	1.9	2.9
5	1.3	2.5	3.2	1.5	2.5	2.4
6	2.3	3.5	2.9	2.4	2.1	2.1
7	1.7	2.1	2.6	1.6	1.2	1.7
Mean±SEM*	1.72±0.17	2.36±0.31	3.02±0.12	1.99±0.21	1.90±0.21	2.43±0.24
0.50 g/kg i.v.						
3	—	2.7	2.3	2.4	2.6	2.8
4	3.2	3.6	3.4	2.7	2.5	3.3
5	2.0	2.9	2.4	2.7	2.3	3.3
6	2.5	2.8	2.9	3.3	2.3	3.1
7	1.9	3.7	4.2	2.2	2.2	2.9
Mean±SEM*	2.39±0.30	3.14±0.21	3.04±0.21	2.64±0.19	2.39±0.64	3.07±0.11
1.00 g/kg i.v.						
3	2.8	4.6	5.5	3.9	3.4	—
4	4.4	3.9	4.7	5.5	2.7	9.0
5	5.1	3.7	5.4	3.8	3.5	7.7
6	3.6	3.9	5.2	2.9	2.8	8.3
7	5.1	4.2	5.9	4.5	4.7	8.6
Mean±SEM*	4.20±0.45	4.06±0.15	5.33±0.19	4.08±0.44	3.40±0.36	8.40±0.27
1.00 g/kg orally						
3	4.2	3.5	3.7	2.5		
4	3.8	3.8	3.5	2.9		
5	3.1	3.9	3.3	1.0		
6	3.3	4.1	3.8	1.1		
7	3.8	4.7	3.3	1.3		
Mean±SEM	3.64±0.20	4.00±0.20	3.50±0.10	1.75±0.40		

* Difference between nitrogen excretion at each level of infusion is not significant when means for T. are compared to those for G. and similarly in the case of K. and S.

During intravenous infusion T. and K. were fed in the order 1.0, 0.5, and 0.25 g/kg, whereas G., W., S., and M. were fed in the order 0.25, 0.5, and 1.0 g/kg.

0.25 g (S., M., G., and W.) or 1.0 g/kg (T. and K.).[2] The fecal nitrogens on oral protein were 1.0, 1.7, 0.11, and 0.09 g/day for patients S., T., G., and W., respectively, which is not above the accepted normal range of 0.8–2.5 g (13), showing good absorption of oral protein.

[2] It was notable that no significant difference in nitrogen excretion could be discerned at each level of infusion when patient T., infused in a sequence of decreasing amounts of amino acid, was compared with patient G., who was of comparable body weight but infused in the reverse sequence of increasing amounts of amino acid. Similar results were obtained by comparing K. with S. (Table V).

Blood amino acid levels. The mean blood amino acid levels at 0900 and 2100 h at each level of amino acid infusion are shown in Table VI.

Change in fasting blood amino acid levels. The fasting blood levels of threonine, glycine, α-amino-*n*-butyric acid, cystine, methionine, isoleucine, tryptophan, and arginine were higher at the 1.0 g than at the 0.25 g/kg treatment level (Table VI). Many of these changes occurred with the onset of nitrogen balance as the input of amino acids was increased from 0.25 to 0.50 g/kg. Threonine was the only amino acid to increase when the infusion rate was increased from 0.5 to 1.0 g/kg.

TABLE VI

Whole Blood Amino Acid Levels

Amino acid intake, g/kg	0.25				0.50				1.00			
	0900 h		2100 h		0900 h		2100 h		0900 h		2100 h	
Amino acid	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
						μmol/100 ml						
Aspartic acid	6.74*	4.19	9.72	6.08	9.84	5.53	11.41	7.42	8.82	3.14	8.09	3.46
Threonine	4.70*	0.74	7.41	1.03	6.04‡	0.64	9.15	1.44	8.09§	0.82	16.23	1.62
Serine	8.57	1.03	8.39	1.25	10.51	1.25	12.38	1.58	11.51	1.28	13.55	1.10
Asparagine	2.89	0.37	3.30	0.48	3.53	0.45	3.65	0.32	3.00	0.37	3.03	0.34
Glutamine	19.32*	2.08	20.74	3.94	25.85	3.32	28.58	4.96	16.21	4.09	24.19	7.98
Proline	7.34*	0.68	10.96	1.48	11.71	2.00	14.00	1.50	9.54	1.60	17.33	1.70
Glutamic acid	11.57	1.38	10.73	0.62	12.74	0.84	13.68	0.74	13.45	2.93	18.42	4.39
Glycine	24.19*	2.17	34.60	4.52	37.24	2.65	53.76	8.64	40.56§	4.88	79.35	7.70
Alanine	22.70*	4.03	36.95	6.68	30.94‖	3.91	45.82	8.42	24.23	4.64	55.36	7.21
α-aminoadipic acid	33.67	4.83	23.56	4.40	29.16	5.04	31.36	5.94	21.84	3.27	32.99	5.62
α-amino-*n*-butyric acid	1.01	0.32	1.59	0.59	1.51	0.20	1.96	0.33	2.56§	1.05	2.31	0.64
Valine	5.34	0.60	7.65	1.18	6.07	0.40	8.04	0.90	6.43	0.60	13.00	1.59
Half cystine	3.32	0.59	3.98	0.92	4.75	0.94	5.42	0.78	5.67§	0.60	6.18	1.30
Methionine	1.40*	0.51	3.43	0.94	3.84	1.17	6.70	1.52	4.06§	0.97	7.06	1.44
Isoleucine	2.30*	0.23	3.92	0.46	3.52	0.39	5.71	0.83	3.60§	0.47	7.76	0.86
Leucine	2.92*	0.36	4.50	0.84	3.57	0.55	5.43	1.04	3.38	0.57	7.24	1.39
Tyrosine	2.42*	0.59	3.00	0.95	2.99	0.54	2.50	0.51	3.28	0.74	3.50	0.89
Phenylalanine	2.68	0.52	3.98	0.72	3.43	0.59	5.48	1.03	3.46	0.63	7.20	1.32
Lysine	6.26	0.65	7.41	1.06	8.03	0.79	9.40	1.77	8.36	0.74	11.03	1.26
Histidine	4.80	0.81	6.77	1.09	4.46	0.71	7.86	0.55	5.62	0.80	9.38	0.67
Tryptophan	0.37	0.16	0.98	0.34	0.50	0.05	1.05	0.43	0.61§	0.15	1.54	0.43
Arginine	2.83	0.69	4.25	0.72	2.72	0.57	7.39	1.18	4.44§	1.03	10.69	1.49

* Significantly less than the 0.50, 0900 h level ($P < 0.05$).
‡ Significantly less than the 1.00, 0900 h level ($P < 0.05$).
§ Significantly greater than the 0.25, 0900 h level ($P < 0.05$).
‖ Significantly greater than the 1.00, 0900 h level ($P < 0.05$).

TABLE VII

Analysis of Change in Blood Amino Acid Concentrations during Amino Acid Infusion

Amino acid	*P* for Difference between Rise at 0.25 and 1.0 g/kg body wt
Threonine	<0.01
Isoleucine	<0.01
Leucine	<0.05
Valine	<0.05
Phenylalanine	<0.05
Tyrosine	N.S.
Methionine	<0.05
Cystine	<0.05
Lysine	N.S.
Tryptophan	N.S.
Glycine	<0.01
Alanine	<0.05
Proline	N.S.
Histidine	N.S.
Arginine	<0.01

Correlation of rise in blood amino acid occurring after 12 h of infusion with the input of amino acid. The blood levels of most of the amino acids increased above the fasting levels at the end of 12 h of infusion (Table VI). In particular, glycine and alanine levels increased, undoubtedly because these were the two amino acids in greatest concentration in the mixture. However, the ease with which the body is able to transfer the nitrogen contained in alanine to other nonessential amino acids is illustrated by its low levels in fasting blood even at the 1 g/kg treatment level.

Individual variability among the patients made consideration of overall changes in the mean blood amino acid levels difficult to assess. This sort of variability was decreased by using the 0.25 g/kg fasting level of each patient as a baseline value and by subtracting this value from the level observed in the blood of each patient after 12 h of infusion with 0.25 or 1.0 g/kg of amino acids. Thus each patient was his own control. With increased rate of infusion (amount of amino acid per 12-h infusion period) there was a statistically significant rise

in the blood levels of the amino acids threonine, isoleucine, leucine, valine, phenylalanine, methionine, cystine, glycine, alanine, and arginine. Of the amino acids infused, tyrosine, lysine, tryptophan, proline, and histidine did not show increased blood levels with increased input (Table VII).

Blood amino acid levels during infusion of amino acids compared to levels observed during oral ingestion of protein. There were many differences between blood amino acid levels measured at 0900 h when the patients were given 1 g/kg of total amino acids intravenously and when given the same amount of mixed protein orally. No simple pattern was apparent in these differences. Aspartic acid, threonine, serine, glycine, alanine, α-amino-*n*-butyric acid, and methionine levels were all lower when the patients were fed protein orally (Table VIII). Valine, cystine, and tyrosine levels were also lower after oral feeding but only in three out of the four instances.

DISCUSSION

The quality of the amino acid mixture used in these studies, as shown by the minimal amount required to promote nitrogen balance, was clearly superior to that of the casein hydrolysate examined previously (1). In that study (1), where six malnourished adults with comparable gastrointestinal disease were infused with casein hydrolysate at levels of 0.25, 0.5, and 1.0 g/kg of body weight, nitrogen retention averaged −2.3, −1.1, and 0.8 g nitrogen/day, respectively. When these values were plotted against input, nitrogen balance (zero nitrogen retention uncorrected for dermal losses) was achieved at 0.8 g casein hydrolysate/kg body weight. By contrast, nitrogen balance in the present study was achieved with 0.4 g amino acids/kg body weight, half that required with casein hydrolysate. This amount was only slightly higher than the 0.34 g/kg estimated to be the requirement for ideal protein to meet the obligatory nitrogen loss of normal healthy adults (2). Feeding 1 g/kg of high quality protein orally, with the remaining nutrients fed intravenously, resulted in a mean nitrogen retention of 2.0 g/day (Table IV), comparable to that seen with the same amount of amino acids given intravenously (mean 2.2 g/day, Table IV).

Earlier work with an identical protocol, had shown that the nitrogen retention with oral cottage cheese and intravenous casein hydrolysate was 3.0 and 0.8 g/day, respectively (1). Since the lower nitrogen retention with casein hydrolysate and the better nitrogen retention

TABLE VIII

Fasting Blood Amino Acid Levels of Four Patients Fed 1 g/kg of Amino Acids Intravenously or Protein Orally

	Patient							
	W.		S.		T.		G.	
	i.v.	Oral	i.v.	Oral	i.v.	Oral	i.v.	Oral
	μmol/100 ml							
Aspartic acid	23.34	1.39	2.98	0.58	5.80	4.24	8.10	5.78
Threonine	8.26	5.02	9.71	1.01	9.62	2.97	8.37	5.81
Serine	11.14	9.99	14.25	9.13	15.19	8.41	11.45	8.53
Asparagine	4.81	1.59	2.66	1.59	2.31	3.75	2.94	3.44
Glutamine	22.02	17.19	4.88	16.16	2.43	17.06	20.85	17.94
Proline	4.80	14.63	13.24	14.62	9.84	8.84	7.59	7.75
Glutamic acid	9.37	13.81	10.62	11.46	13.27	10.47	10.71	9.28
Glycine	32.76	23.10	42.42	22.79	57.73	21.61	32.11	20.65
Alanine	15.62	13.57	42.01	29.45	18.56	14.22	18.10	15.39
α-aminoadipic acid	7.26	22.36	21.48	19.59	28.74	26.71	19.66	29.26
α-amino-*n*-butyric acid	7.59	trace	2.58	trace	1.04	0.77	2.12	1.87
Valine	7.88	5.11	7.00	1.19	7.10	6.14	6.24	6.19
Half cystine	5.89	1.15	5.92	0.42	5.32	5.43	6.11	5.26
Methionine	5.90	1.62	5.87	1.45	5.84	2.62	4.56	3.64
Isoleucine	3.89	4.44	5.28	1.10	3.62	2.19	3.49	3.61
Leucine	5.31	5.39	3.47	1.06	4.25	3.59	3.32	3.11
Tyrosine	6.20	2.55	3.23	0.72	3.22	2.81	4.08	3.91
Phenylalanine	5.91	2.05	4.46	0.65	3.12	4.15	3.03	3.06
Ornithine	4.96	11.05	6.54	3.60	5.36	3.71	7.23	5.58
Lysine	10.27	12.64	7.67	7.60	7.49	6.43	9.07	8.80
Histidine	4.28	9.04	5.17	2.24	5.71	5.02	9.40	3.83
Arginine	3.58	7.54	7.48	2.68	3.27	1.37	7.49	5.44

TABLE IX

Essential Amino Acid Requirements of Children and Adults Compared to the Amino Acids Supplied

Amino acid	Requirements*		Amino acids supplied‡			Casein hydrolysate‖
	Adults	Children 10–12 yr	0.25	0.5	1.0	1.0
			mg/kg			
Isoleucine	10	28	12.0	24	48	44
Leucine	13	49	15.5	31	62	82
Methionine and cystine	13	27	14.5	29	58	27
Phenylalanine and tyrosine	14	27	16.5	33	66	52
Threonine	6	34	10.5	21	42	48
Tryptophan	3	4	4.5	9	18	9
Valine	13	33	11.5	23	46	57
Lysine	10	59	14.5	29	58	73
Total essential	82	261	99.5	199	398	392
Total nonessential	343	439	150	301	602	608
Total amino acids	425	700	250	500	1,000	1,000
Essential/Total, %	19	37	40	40	40	39

* From Munro (2).

‡ From amino acid mixture: amount of amino acids supplied when mixture is infused at 0.25, 0.5, and 1.0 g/kg body weight.

‖ Reported as free essential amino acids. Total nonessential amino acids are reported as the sum of peptide-bound and nonessential amino acids (1).

with the defined amino acid mixture were demonstrated in different groups of patients, it is possible to explain the difference in nitrogen balance as due either to a difference in the disease process or to the quality of the mixture infused. A comparison of nitrogen retention in the two groups when fed high-quality protein orally supports the latter conclusion. When high quality protein was fed orally to either group, with an identical protocol, the mean nitrogen retention was comparable, demonstrating that the groups were capable of retaining comparable amounts of nitrogen with a reference protein intake. The results of intravenous versus oral protein can be compared, despite the fact that when meat and eggs were fed orally the patients received some lipid calories, while during intravenous administration the amino acids were given with glucose as the only concurrent source of nonnitrogenous calories. Such a comparison is valid because studies [3] have been done in random order comparing the nitrogen balance observed when glucose supplied all of the nonprotein calories with that when 83% of the nonprotein calories came from lipid and 17% from glucose (when the total amount of amino acids infused was kept constant). These studies showed that nitrogen balances for the two groups were 1.0±0.60 g and 1.4±0.57 g, respectively, values that are not significantly different ($P > 0.5$).

In a previous study (1) that correlated the lower nitrogen retention observed during infusion of casein hydrolysate (lower than that seen with oral cottage cheese) with changes in blood amino acid levels, it had been seen that blood cystine plus methionine and phenylalanine plus tyrosine levels did not increase during infusion of casein hydrolysate. Since the provision of sufficient amino acids to exceed requirements should have resulted in an expansion of the circulating amino acid pool during infusion, it was concluded (1) that the hydrolysate was deficient in sulphur-containing (methionine and cystine) and aromatic (phenylalanine and tyrosine) amino acids. Upon administration of the present mixture, which does contain greater amounts of these two groups of amino acids (Table IX), a rise in blood levels of these amino acids would be expected with the onset of nitrogen balance. An inspection of Tables VI and VII indicates that both methionine and phenylalanine concentrations significantly increased during infusion (Table VII), supporting our theoretical prediction. The only two essential amino acids that did not increase were lysine and tryptophan. Considering lysine first, it is apparent from Table IX that among the amino acids, the lysine requirement is the only one below that for a 10–12-yr-old child, while all other essential amino acids provide between 1.2 and 4.5 times the requirements, if 1.0 g/kg body wt of the mixture is infused daily. The amino acid requirements of the patients were compared to those of a child because of the argument put forth by Munro that the needs for essential amino acids in the malnourished adult may be increased and become equivalent to those of a child (2). Hence, in relation to the

[3] Sanderson, I., and K. N. Jeejeebhoy. Unpublished results. results.

requirements for essential amino acids, lysine was provided in this mixture in the lowest proportion. Correspondingly, no rise in blood lysine was observed during infusion of the mixture, a finding in accordance with its relative availability. It is of interest that casein hydrolysate provides more lysine than this mixture and a significant rise in lysine was noted during infusion of that hydrolysate (1).

Second, tryptophan blood levels were relatively constant in this study and in the previous study, in which casein hydrolysate was the amino acid source. Yet the tryptophan supply should have been more than adequate (Table IX), since the minimal intake of tryptophan was 4.5 mg/kg. Young et al. (9) found that plasma levels of free tryptophan were unchanged above intakes of 5 mg/kg and that the adult requirement for tryptophan was only 3 mg/kg. With intakes above 5 mg/kg, perhaps hepatic tryptophan oxygenase is induced, resulting in increased catabolism of the amino acid and a constant blood amino acid level.

It might be expected from the study of Young and his associates, in which they have estimated the tryptophan (9) and lysine and valine (10) requirements of young men, that fasting levels of the essential amino acids would increase when the requirements for these amino acids were met. However, in their studies the dietary concentration of the amino acid under test was increased, while the total amino acid content was kept constant. In the present studies, although the fasting blood levels of threonine, methionine, isoleucine, leucine, and tyrosine rose between the 0.25 and 0.5 g/kg treatment levels, concurrent with a change from negative to positive nitrogen balance, the levels of valine, cystine, phenylalanine, lysine, and tryptophan did not change (Table VI). This indicated that fasting levels did not rise with attainment of positive nitrogen balance in many essential or semi-essential amino acids. Perhaps protein synthesis and/or protein catabolism is uniformly stimulated when the total input of amino acids is increased (14). There was better correlation between the rise in blood amino acids during infusion and their requirement on theoretical grounds. Furthermore, blood amino acid levels during infusion varied in the way predicted from experimental manipulation of the mixture (and from comparing casein hydrolysate with this mixture). These observations suggest that the degree of elevation of blood amino acid levels in response to a defined rate of graded infusions appears to be a good measure of the amino acid balance in a mixture used for parenteral alimentation. The latter conclusion is supported by the findings of Graham, Baertl, and Placko, (15), who demonstrated that postprandial levels are superior to fasting levels for determining adequacy of amino acids in the diet.

TABLE X

Normal Fasting Whole Blood Free Amino Acid Concentration

Amino acid	Literature Mean*	Laboratory Mean‡	Laboratory SEM‡
Taurine	13.38	16.36	2.19
Aspartic acid	10.56	12.11	2.64
Threonine	14.20	12.00	1.09
Serine	20.86	10.90	0.61
Asparagine	—	6.08	0.24
Glutamine	25.02	26.25	1.91
Proline	—	14.40	1.90
Glutamine acid	12.16	11.84	0.50
Citrulline	2.56	3.86	0.24
Glycine	23.08	22.98	2.99
Alanine	25.48	25.53	1.95
α-aminoadipic acid	—	20.89	3.53
α-amino-*n*-butyric acid	1.64	4.11	0.21
Valine	15.32	15.74	1.36
Half cystine	5.26	4.71	0.64
Methionine	1.88	3.56	0.32
Isoleucine	4.66	6.09	0.34
Leucine	14.84	9.89	0.47
Tyrosine	7.62	6.53	0.44
Phenylalanine	6.04	6.36	0.28
Ornithine	13.70	10.72	0.92
Lysine	15.56	15.02	0.93
Histidine	11.62	10.09	0.30
Arginine	4.30	9.07	0.49

* Calculated from Levy and Barkin (16) with a hematocrit value of 40%.

‡ Blood samples obtained between 0900–1000 h from five healthy adults after an overnight fast.

Although whole blood rather than plasma was prepared for amino acid analysis in the studies presented, it is unlikely that this factor accounted for our inability to reproduce the observations of Young et al. (9, 10). Snyderman *et al.* (5) have shown that increasing protein intake causes a proportionate rise in the free amino acids of plasma and red blood cells. Thus the overall results of looking at plasma, red blood cells, or whole blood concentration of many of the amino acids might be expected to be the same. The concentrations of most of the essential amino acids are similar in plasma and erythrocytes, except for methionine and cystine, which are lower in erythrocytes (16). Therefore the levels reported in Table VI for these sulfur amino acids are lower than those expected in plasma. However, the fasting essential blood amino acid levels are lower than expected. Possibly the supply of energy in the form of lipid throughout the night did not allow the amino acids to adjust to a true fasting state. Thus protein synthesis may have continued, and/or protein catabolism decreased, and this may have contributed to the

uniformity of the blood amino acid levels over the range of input studied in these experiments as well as the generally low levels observed. These low levels observed in the patients were not due to the method used, since the results in healthy controls were comparable in most instances to those obtained by Levy and Barkin (16) (Table X).

Most of the nonessential nitrogen was distributed among the amino acids alanine, glycine, and proline. The blood levels of these amino acids increased during infusion. However, the 12-h postinfusion levels of proline and alanine were normal (Table VI), indicating that the nitrogen was readily utilized or distributed to other nonessential amino acids. On the other hand, glycine levels tended to remain elevated. Although it has been reported that loss of glycine in the urine occurs when large amounts of this amino acid are provided by a parenteral mixture (17), it is unlikely that much loss occurred in these studies where as little as 0.5 g/kg of amino acids maintained a nitrogen balance of 0.5 g/day.

In conclusion, blood aminograms, utilized in the manner described and combined with nitrogen balance studies, can be used effectively to study the balance of the amino acids in the mixture. Furthermore, the studies have shown that the utilization of a well-balanced amino acid mixture delivered intravenously can be as efficient as protein given orally.

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